Emulsion Stability

Nicomp® and AccuSizer®

EMULSION STABILITY

Most emulsions are not naturally stable and require careful formulation to create dispersions with enhanced shelf life. Various theories and instrumental techniques are available to help formulators choose the optimum chemistry to achieve desired results. This application note is not a guide to emulsion formulation, but rather an introduction to the analytical techniques available to guide the study of how to create stable emulsions.

An emulsion is a mixture of two, or more liquids, that are not typically miscible. Most are a two-phase system with a dispersed phase (smaller volume) and a continuous phase (greater volume). Types of emulsions include oil in water (o/w), water in oil (w/o), and double emulsions such as a water in oil in water (w/o/w) emulsion. In an o/w emulsion the dispersed phase is the oil and the continuous phase is the water.

Creating an emulsion typically requires an energy source to form the emulsion such as shaking, stirring, ultrasound, homogenizer, or microfluidizer.[1] Most emulsions destabilize over time, sometimes immediately after the energy input has ceased. Chemicals known as emulsifiers are added to extend the stable period and delay phase separation.

Emulsifiers are typically surfactants containing a hydrophilic head and a hydrophobic R-C chain. The hydrophobic tail orients towards the organic phase, and the hydrophilic head orients towards the water. By positioning itself in this orientation at the interface the emulsifier reduces the surface tension and increases the charge (the zeta potential) on the droplet surface, resulting in a stabilizing influence on the emulsion, see Figure 1. Types of emulsifiers include food products such as lecithin, sodium phosphates, and surfactants (both ionic and non-ionic). Viscosity modifiers, such as PEG, can also be added to increase emulsion stability.

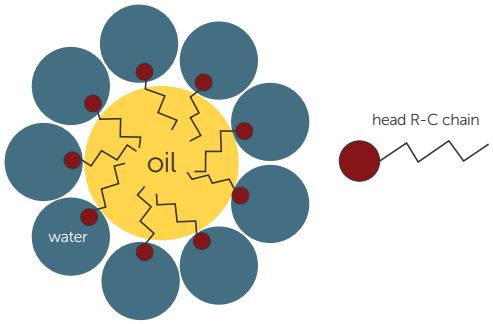

Figure 1. Emulsion with surfactant

EMULSION FORMULATION AND STABILITY STUDY

A combination of analytical techniques was used to investigate emulsion formulation and stability. Two surfactants, at varying concentrations, were used to create oil in water emulsions. The mean size of the emulsion droplets was determined using the Nicomp® dynamic light scattering (DLS) system.

The Nicomp was also used to measure the zeta potential of the droplets for all samples. Zeta potential can be used as a predictor of dispersion stability. The results from the Nicomp DLS measurements are reported as mean size and polydispersity index, PI.[2]

The AccuSizer® single particle optical sizing (SPOS) system was used to measure the large diameter droplet tail, an indication of emulsion stability. The relationship between large diameter droplet tail as measured on the AccuSizer and emulsion stability is well documented[3,4] and has been codified into the pharmaceutical test USP<729> Globule size distribution in lipid injectable emulsions.[5,6] In USP<729> the volume percent greater than 5 µm (PFAT5) is used as the indication of emulsion stability, with a limit of 0.05%. USP<729> also calls for use of DLS to determine the mean droplet size of emulsions with a limit of less than 500 nm for the intensity mean diameter.

While the DLS mean diameter, zeta potential, and large diameter droplet tail are all indicators of emulsion stability, the formulaction Turbiscan[7] is a direct measurement of dispersion stability. The Turbiscan can detect particle migration and size change in order to quantify destabilization phenomena; in this case, the phase separation (creaming) of the emulsion as a function of time. A sample is placed in the Turbiscan, and an NIR light sources scans both transmission and backscatter up and down the height of the sample bottle (Figure 2). Since the emulsions in this study were fairly opaque the backscatter data was used to characterize the samples.

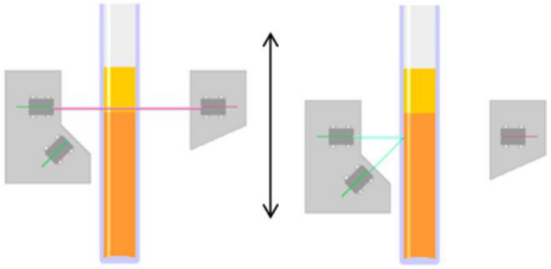

Figure 2. Turbiscan transmission (left) and backscatter (right) detectors

These techniques were used to analyze the emulsion samples at the time of creation, and as a function of time over 15 minutes and several hours. A more complete study of emulsion stability would take much longer than the time frame chosen for this study, the goal of this study was to show simply how the instruments and data can be used. This study was not intended to be a reference guide on emulsion formulation and/or long term stability analysis.

Several oil in water emulsions were created to study stability. All emulsions were created by mixing 1 mL of mineral oil into 19 mL of DI water containing a surfactant. Two surfactants were used at two concentrations:

A: Anionic Surfactant and Emulsifier

- A High: 10 g dissolved in 100 mL DI water
- A Low: 2.5 g dissolved in 100 mL DI water

B: Nonionic Surfactant and Emulsifier

- B High: 5 mL in 100 mL DI water
- B Low: 1 mL in 100 mL DI water

In all formulations the surfactant was added to the water, stirred for 10 minutes and raised to 50°C. The mineral oil was raised to 40°C, and then added to the water/surfactant solution. The oil/water mixture was next sonicated for two minutes using an ultrasonic probe.

INSTRUMENTATION

The particle size was measured by two techniques:

- Dynamic light scattering (DLS) using the Nicomp Z3000 for submicron particle size and zeta potential
- Single particle optical sizing (SPOS) using the AccuSizer 780 APS for particle size 0.5 – 400 µm

Using two techniques to measure the size and stability of emulsions is a well documented approach and incorporated into the pharmaceutical USP test<729> for lipid emulsions.[6]

The emulsion stability was measured using the Formulaction Turbiscan.

INSTRUMENT SETTINGS

DLS size: The mean size and PI was measured on the Nicomp using the settings shown below:

- Channel width: automatic; typical value was 38 µs
- Temperature: 23°C
  (let peltier cool sample before analysis)
- Liquid viscosity: 0.933 c
- Intensity setpoint: automatic
- Laser wavelength: 658 nm
- Measurement angle: 90°
- Cell type: disposable square cuvette
- Baseline adjust: automatic
- Algorithm: Gaussian

Zeta potential: The zeta potential measurements were programmed using the setup conditions shown below:

- Temperature: 23°C
- Liquid viscosity: 0.933 cP
- Scattering angle: -14.14°
- Dielectric constant: 78.5
- Cell type: dip cell in square cuvette
- Electrode spacing: 0.4 cm
- E-Field strength: 4 V/cm
- ka: Smoluchowski
- Analysis type: PALS (not constant current)

SPOS size: The AccuSizer APS measurements were programmed using the setup conditions shown below:

- Data collection time: 60 sec
- Number channels: 128
- Diluent flow rate: 60 mL/sec
- Target concentration: 4500 part/mL
- Background threshold: 100 part/sec
- Sensor: LE400
- Calibration: summation mode
- Injection loop: 0.5 mL
- Syringe volume: 1 mL
- Sample flow time: 5 sec
- Initial DF2: 1200

Stability. Measurements were programmed using the setup conditions shown below:

- Measurement time: 15 minutes*
- Scan rate: every 30 sec
- Temperature: 40 C
- Data reporting: backscatter and TSI (global)**

**This is a very short measurement time that would typically be extended in such studies.*

***The Turbiscan stability index (TSI) is a one-click calculation that compares the variances in the signals from scan to scan. A high TSI means that there are a lot of variances in the scans and therefore a lot of particle movement/size increase and an unstable sample. A low TSI is just the opposite - there are very few variances in the scans and therefore a more stable emulsion.*

RESULTS

The easiest place to begin understanding the collected data is to consider Figure 3 that shows the destabilization kinetics value of TSI (global) as a function of time over 15 minutes for the four samples.

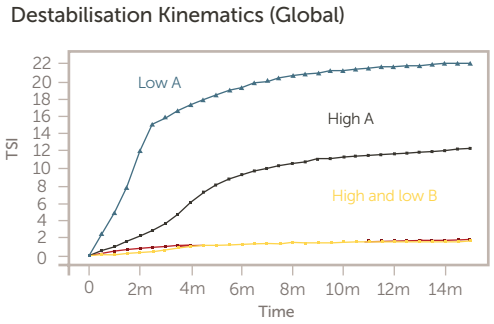

Figure 3. Turbiscan TSI plots for all samples

These results indicate the following:

- Low A and High A were both extremely unstable emulsions, with High A being more stable than Low A.
- Low B and High B were much more stable emulsions, with High B being slightly more stable than High B.
- Individual backscatter results for these samples are shown in Figures 6 – 10 for better visual interpretation of the data. Low A and High A suffer from very large creaming phenomena at the top of the vial, whereas High and Low B suffered from only slight particle movement and size change.

Another easy way to understand the collected data is to look at the volume distribution from the AccuSizer APS system shown in Figure 4.

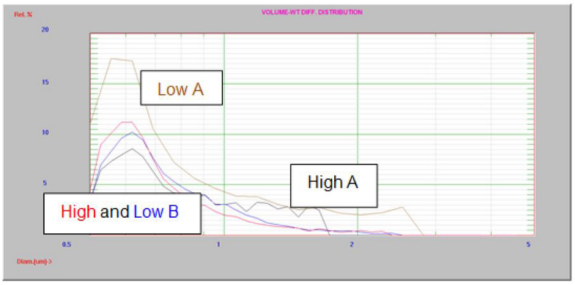

Figure 4. Relative volume % distributions

It is visually apparent that the order of decreasing percentage of large diameter tails is Low A> High A> Low B> High B. This tracks the Turbiscan results seen in Figure 3.

As documented in references[3,4,5] a higher volume percentage of large diameter droplets indicates a less stable emulsion. In USP <729> the percentage greater than 5 µm was chosen as the value to set specifications on. The AccuSizer results shown in Figures 4 were generated within 10 minutes of preparing each emulsion, with no droplets yet appearing in the greater than 5 µm range. Therefore, a value, such as volume percent greater than 1 µm, might be a better calculation to focus on to differentiate these samples at the initial creation time. Sample High A was analyzed again four hours after the result shown in Figure 4. Figure 5 shows that the droplets have dramatically increased in size and now there is a distinct population found greater than 5 µm.

Figure 5. High A 10 minutes (blue) and 4 hours (red) after creation

It is important to realize that the AccuSizer results shown in this document do not represent the entire distribution, only the large tail. The LE400 sensor used for this study has the dynamic range of 0.5 — 400 µm, therefore, the vast majority of the droplets are below the detection limit. This is why DLS was used to determine the mean size of the emulsion distributions. The DLS mean size, PI, and zeta potential of the samples analyzed is shown in Table 1.

Sample	DLS size	PI	Zeta potential
Low A	350.1	0.55	-47.2
High A	301	0.207	-61.18
Low B	292.6	0.379	-24.55
High B	283.3	0.229	-30.88

Table 1. DLS size and zeta potential results

The smaller mean size for surfactant B suggests a more stable emulsion, as indicated by the Turbiscan data shown in Figure 3 and AccuSizer data in Figure 4. For both A and B a higher surfactant concentration resulted in smaller size and PI value, and a higher zeta potential value. But the fact that both zeta potential values for A are greater than B shows that zeta potential alone, does not settle the question of optimum formulation conditions. The more important consideration is which surfactant is actually a better emulsifier for a given emulsion type. The hydrophile-lipophile balance (HLB) is an empirical expression for the relationship between the hydrophilic and hydrophobic parts of a surfactant.[8] Applying the HLB calculations to a given emulsion formulation often provides greater insight into surfactant choice and expected emulsion stability. In general, o/w emulsions require higher HLB surfactants and w/o emulsions require lower HLB surfactants.

CONCLUSIONS

Each of the instruments used in this study provided useful information to guide the formulation and stability analysis of the emulsions investigated. The Formulaction Turbiscan is a direct measurement of emulsion stability and provided easy to interpret data quantifying the relative stability of the emulsions. Emulsions Low and High A were intentionally created to be very unstable in order to accentuate the ability of the Turbiscan to quantify the differences very quickly. In real practice the Turbiscan measurements would typically require a longer time scale than used in this brief study.

The Nicomp provided quick, easy mean droplet size and zeta potential data, generating excellent initial information for the emulsions studied. Greater zeta potential is an indicator of greater stability. But most emulsions just require some zeta potential value greater than at least 10 mV to achieve some level of stability. Looking at zeta potential alone will not answer all questions regarding the stability of a range of formulations when more than one surfactant is involved.

The AccuSizer generated quick, unambiguous data to predict and track emulsion stability. This is the standard technique used to determine lipid emulsion stability in the pharmaceutical industry and should see wider usage in general emulsion formulation studies.

ADDITIONAL RESULTS

Backscattering - LS1

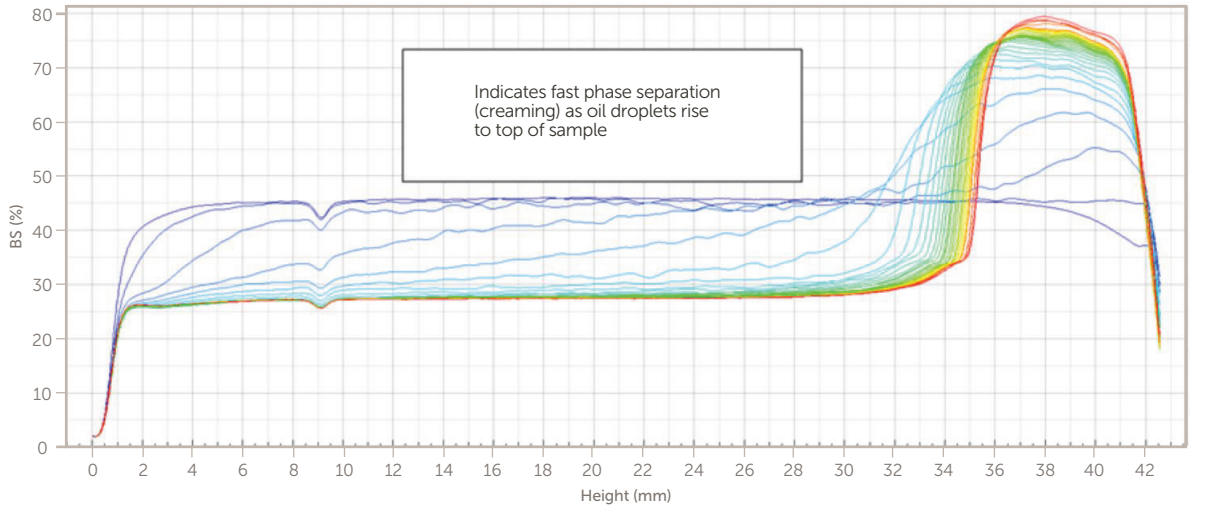

Figure 6. Turbiscan backscatter result for Low A

Backscattering - LS1

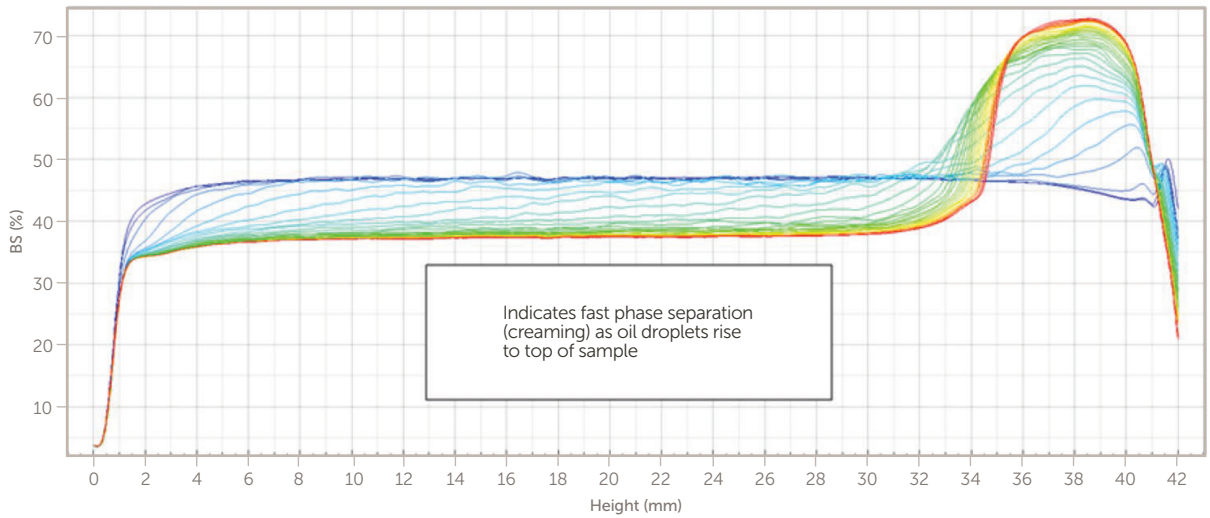

Figure 7. Turbiscan backscatter result for High A

Backscattering - LS1

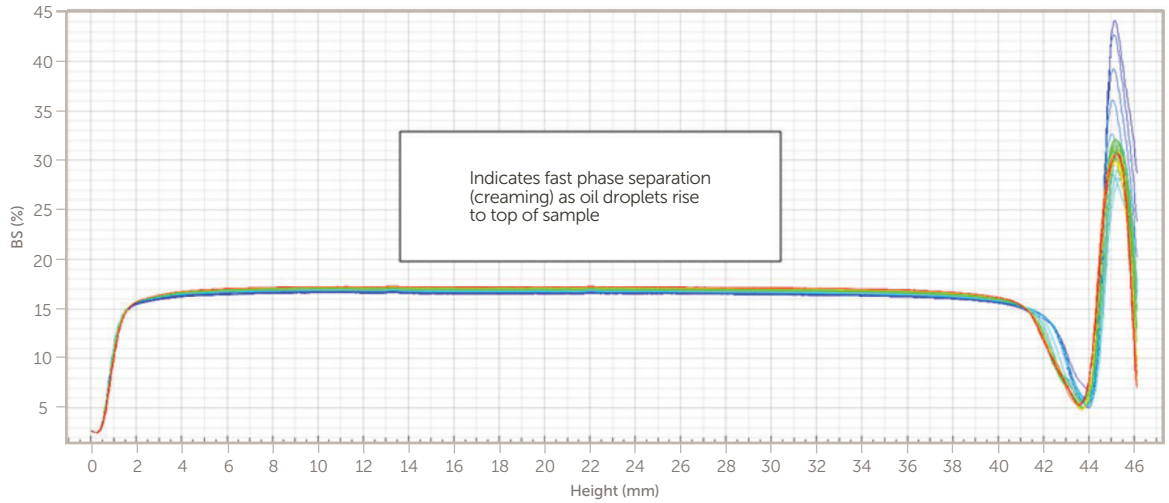

Figure 8. Turbiscan backscatter result for low B

Backscattering - HT1

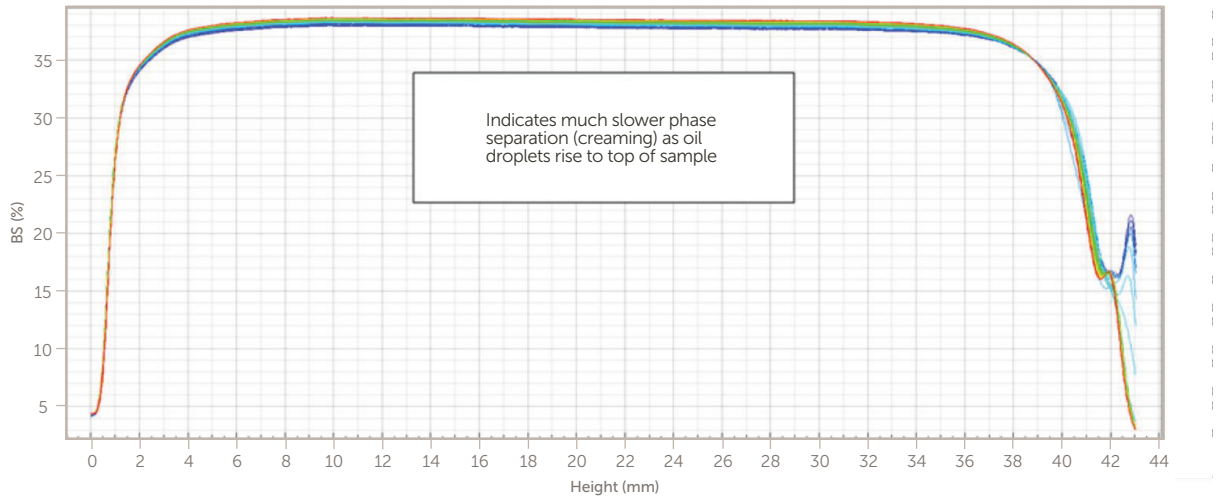

Figure 9. Turbiscan backscatter result for high B

Destabilisation Kinetics (Global)

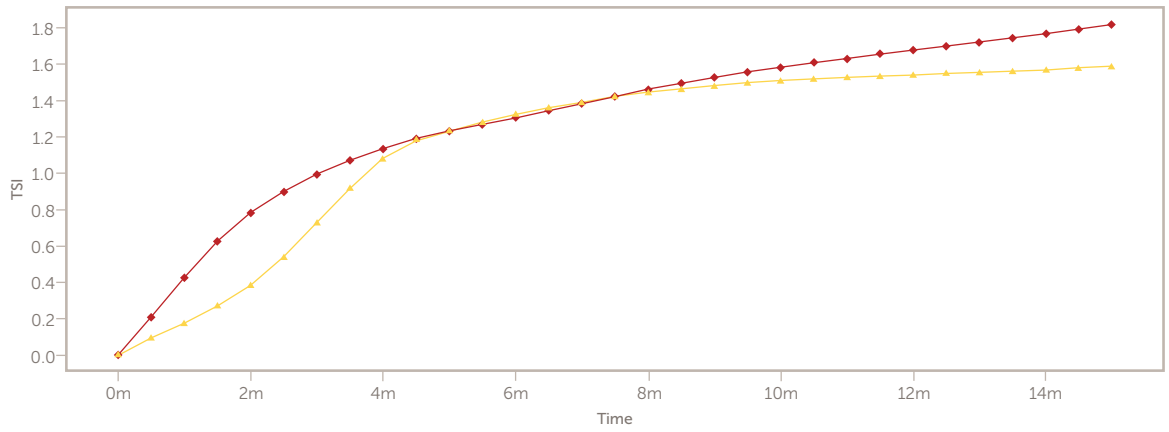

Figure 10. Turbiscan TSI global result low B (red) high B (yellow) enlarged to show differences

References

[1] Entegris Application Note - Size Reduction by a Microfluidizer

[2] Entegris Technical Note - DLS Data Interpretation

[3] Driscoll, D. et. al., *Physicochemical assessments of parenteral lipid emulsions: light obscuration versus laser diffraction,* International Journal of Pharmaceutics 219 (2001) 21–37

[4] Driscoll, D. et. al., *Fat-globule size in a propofol emulsion containing sodium metabisulfite,* Am J Health-Syst Pharm – Vol 61 Jun 15, 2004

[5] USP <729>, Globule Size Distribution in Lipid Injectable Emulsions

[6] Entegris Application Note - USP <729>

[7] Formulaction, http://www.formulaction.com/

[8] Vaughan, C.D. Rice, Dennis A.; Predicting O/W *Emulsion Stability by the Required HLB Equation*; Journal of Dispersion Science and Technology; 1990. Vol. 11 (1), pp 83 – 91

FOR MORE INFORMATION

Please call your Regional Customer Service Center today to learn what Entegris can do for you. Visit entegris.com and select the Contact Us link to find the customer service center nearest you.

TERMS AND CONDITIONS OF SALE

All purchases are subject to Entegris' Terms and Conditions of Sale. To view and print this information, visit entegris.com and select the Terms & Conditions link in the footer.

Entegris

Corporate Headquarters
129 Concord Road
Billerica, MA 01821
USA

Customer Service
Tel +1 952 556 4181
Fax +1 952 556 8022
Toll Free 800 394 4083